Comm.
Dr. Gardner
Dr. Huebner
Dr. Jacobson

EPIDEMIOLOGY

#941-Pt. II

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8585

NOV 9 1973

Application for Research Grant
(Use extra pages as needed)

Date:

1. Principal Investigator (give title and degrees):

Henry T. Lynch, M.D., Professor and Chairman, Dept. of Preventive Med. and Public Health
Ibert C. Wells, Ph.D., Professor and Chairman, Dept. of Biological Chemistry
Hoda Guirgis, Ph.D., Assistant Professor, Dept. of Preventive Med. and Public Health

2. Institution & address:

Creighton University School of Medicine
2500 California Street
Omaha, Nebraska 68178

3. Department(s) where research will be done or collaboration provided:

Department of Preventive Medicine and Public Health
Department of Biological Cehmistry

4. Short title of study:

Aryl hydrocarbon hydroxylase (AHH): Cancer genetics

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

Inducibility of aryl hydrocarbon hydroxylase (AHH) will be measured in lymphoblasts from patients from low and high risk cancer prone families in order to determine familial patterns of AHH induction susceptibilities (low, medium, and high). Possible associations between cancer risk and the inducibility of AHH will be correlated with specific histologic varieties of cancer and their genetic modes of transmission. Intensive tumor and genealogic documentation will permit critical appraisal of the significance of AHH findings.

The association of AHH induction susceptibility with other factors, e.g. smoking history, drug consumption, environmental exposures to carcinogens including occupational carcinogens and cancer history will be studied.

As a continuation of this study, it will be of interest to investigate as possible markers other enzymes that are simultaneously induced in lymphoblasts by various carcinogens.

8. Brief statement of working hypothesis:

Cancer prone and cancer free families, when appraised with a high degree of validity and reliability through histologic verification of cancer and precise genealogy, provide valuable resource material in the quest for biochemical markers indicating cancer risk. AHH, having shown increased inducibility in patients with carcinoma of the lung, and possibly in adenocarcinoma of the colon, merits testing in well-defined human clinical cancer genetic problems. If it could be demonstrated that patients at high genetic risk for cancer also have concommitant high susceptibility for AHH inducibility (the reciprocal for low cancer genetic risk patients), then we would have a potentially valuable experimental test for the study of carcinogenesis in man. This would also provide additional diagnostic information, particularly when coupled with cancer genetic risk, and could be utilized in cancer control programs. In summary, we would suspect that the genetic aspect of carcinogenesis might be concerned with the inducibility of AHH and/or other mixed function oxidases; chemical carcinogenesis will then depend upon the conversion of potential carcinogens into active carcinogens, by these mixed function oxidases. Our hypothesis will, therefore, be tested in clinical models noteworthy for genetic susceptibility or resistance to cancer.

9. Details of experimental design and procedures (append extra pages as necessary) (See Appendix for literature review)

Aryl hydrocarbon hydroxylase (AHH) inducibility will be determined in lymphoblasts by measuring AHH activities of cells exposed to 3-methylcholanthrene and dibenzanthracene and comparing these activities with those determined in control cells not exposed to these materials. The procedure to be employed is a modification of the fluorometric procedure of Kellerman et al (18). Fifteen to 20 ml. of heparinized blood will be collected from each patient and total lymphocytes will be separated using 4% dextran solution. These cells will then be incubated in culture media containing phytohemagglutinin, pokeweed mitogen, heparin and fetal calf serum at 37°C. for 72 hours. 3-methylcholanthrene and dibenzanthracene will be added separately to duplicate cultures and incubation will be continued for another 24 hours. Culture media will be removed from all cell cultures and replaced with buffer medium containing NADH, NADPH and 3,4-benzpyrene. Incubation will be continued for 35 minutes and the enzymatic reaction then will be stopped by the addition of 25% acetone in hexane which will also extract the reaction product (3-hydroxybenzpyrene) from the buffer solution. The hexane solutions will then be extracted with 1 N NaOH which selectively removes the reaction product from the NaOH insoluble benzpyrene. The amount of reaction product in each NaOH extract will be determined fluorometrically using an Aminco-Bowman spectrofluorometer with excitation at 396 nm. and emission at 522 nm. Amounts of 3-hydroxybenzpyrene formed will be expressed for comparison purposes, per 3×10^6 cells per 35 minutes. AHH inducibility will than be expressed as the ratio of the amount of 3-hydroxybenzpyrene formed in cells exposed to 3-methylcholanthrene or dibenzanthracene to the amount of 3-hydroxybenzpyrene formed by identical cells not exposed to these hydrocabons.

AHH inducibilities (as well as AHH levels) will be determined on probands and selected relatives so that genetic cancer risk, determined by pedigree analysis, will provide a testable experimental parameter. The following groups will be studied: Group A, 200 probands having no history of cancer, their spouses and their children; Group B, 100 probands having one first degree relative with cancer, their spouses and their children; Group C, 100 probands having 2 or more first degree relatives with cancer, their spouses and their children; and Group D, 200 relatives from cancer-prone and 200 relatives from cancer-free lines of families with the cancer family syndrome. These groups will provide a population sample of about 2000 individuals. Blood samples will be collected in the field and the samples will be transported to Omaha and processed within 12 hours of collection.

Standard statistical methods will be used to test significance of correlations.

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

A laboratory with the usual equipment for biochemical research such as pH meters, colorimeters, centrifuges, etc. is available together with the following special equipment: Beckman quartz spectrophotometer, Amino-Bowman spectrofluorometer with photo multiplier microphotometer and strip chart recorder, refrigerators, deep freezers, Amino refrigerated bath, Virtis freeze-dry apparatus, refrigerated low-speed centrifuge (International HR-1), Spinco preparative ultracentrifuges (L and L2-65), Spinco analytical ultracentrifuge (Model E) with schlieren and ultra violet optics, walk-in refrigerated room, Dubnoff incubator, Warburg apparatus, autoclave, a variety of chromatography equipment including that for paper, columns, thin-layer and gas-liquid, LKB fraction collector, a variety of electrophoresis equipment including that for paper, cellulose strip, starch gel, agarose and disc (both analytical and preparative) using polyacrylamide gels, and LKB immuno-electrophoresis equipment. A Beckman amino acid analyzer (Model 120 B) is also available together with the ancillary equipment necessary for the amino acid analysis of proteins. In addition, there is an autoanalyzer (Technicon) for use in the assay of column eluates for peptides, and an isotope laboratory which contains the usual facilities for handling labeled compounds together with the following special instruments: scaling unit, automatic sample changer and windowless flow counter (Q-gas counter), strip counters (Nuclear-Chicago and Packard), radioactivity survey meter and three-channel liquid scintillation spectrometer with automatic background substract and calculator (Nuclear-Chicago). A complete facility for tissue culture is available including incubators, aseptic work areas, etc. Finally there are available well-kept and supervised animal quarters. A 27 ft. Winnebago motor home modified to include necessary laboratory facilities (centrifuges, etc.) will be used for sample collection.

11. Additional facilities required: None

12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).
Reprints not available.

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CURRICULUM VITAE

NAME: Henry T. Lynch S.S. NUMBER: REDACTED

PLACE AND DATE OF BIRTH: REDACTED

PRESENT ADDRESS: Department of Preventive Medicine
and Public Health
The Creighton University
School of Medicine
Omaha, Nebraska 68178

MARITAL STATUS: REDACTED

EDUCATION:

B.S. R University of Oklahoma, Norman

M.A. R Denver University, Denver

REDACTED University of Texas, Austin
Work toward Ph.D. in Human Genetics
Major field: Human Genetics
Minor field: Biochemistry
Psychology
Course work completed. Dissertation was in progress on admission to Medical School

M.D. R University of Texas Medical Branch, Galveston

St. Mary's Hospital, Evansville, Indiana
Rotating Internship completed

REDACTED University of Nebraska College of Medicine
Residency in Internal Medicine completed

REDACTED A "Short Course in Medical Genetics," supported by the National Foundation; Coordinated by Dr. Victor McKusick, Bar Harbor, Maine, August 3-14

REDACTED Senior clinical cancer trainee, U.S.P.H.S., Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska College of Medicine, Omaha, Nebraska

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1962-65	Medical Genetics Consultant and Lecturer, Department of Orthodontics, Dr. Sam Weinstein, Chairman, University of Nebraska College of Dentistry, Lincoln, Nebraska.
1962-64	Lecturer in Human Genetics, Graduate and Undergraduate students, Department of Zoology, Dr. Dwight Miller, Chairman, University of Nebraska, Lincoln, Nebraska.
1964-66	Instructor, Internal Medicine; Senior Cancer Trainee, U.S.P.H.S., University of Nebraska College of Medicine and Eppley Institute for Research in Cancer and Allied Diseases, Henry M. Lemon, M.D., Director.
6/1/66-10/67	Assistant Professor of Biology, Department of Biology, Assistant Internist, Department of Medicine, Section of Human Genetics, the University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.
10/67	Associate Professor and Chairman, Department of Preventive Medicine and Public Health, The Creighton University School of Medicine, Omaha, Nebraska.
6/1/68	Assistant Professor, Department of Medicine, Creighton University School of Medicine.
9/1/70	Professor & Chairman, Dept. of Preventive Medicine and Public Health, The Creighton University School of Medicine, Omaha, Nebraska.
1/72	Subcommittee on Epidemiology of the Breast Cancer Task Force National Cancer Institute, Bethesda, Maryland

MEMBERSHIP IN SCIENTIFIC SOCIETIES:

REDACTED

REDACTED

REDACTED

Personal Publications (five most recent)

Lynch, H.T., Krush, A.J., Lemon, H.M., Kaplan, A.R., Condit, P.T., and Bottomley, R.H.: Tumor Variations in Families with Breast Cancer, J.A.M.A. 222:1631-1635, 1972.

Lynch, H.T., Krush, A.J., and Kaplan, A.R.: Cancer Frequency Variations Among and Within Families, Acta Genet. Med. Gemellol. 21:53-65, 1972.

Lynch, H.T., Guirgis, H.A., Swartz, M.W., Lynch, J.S., Krush, A.J., and Kaplan, A.R.: Genetics and Colon Cancer, Arch. Surg. 106:669-675, 1973.

Lynch, H.T., Krush, A.J., Harlan, W.L., and Sharp, E.A.: Association of Soft Tissue Sarcoma, Leukemia, and Brain Tumors in Families Affected with Breast Cancer, Amer. Surg. 39:199-206, 1973.

Lynch, H.T., Lynch, J., and Kraft, C.: A New Approach to Cancer Screening and Education, Geriatrics 28:152-157, 1973.

Lynch, H.T., Kaplan, A.R., Moorhouse, A., Krush, A.J., and Clifford, G.: Dermatoglyphic Peculiarities in Members of a High-Cancer-Risk Kindred, Oncology, in press.

A. Co-Principal Investigator: Ibert C. Wells, Ph. D.

Biographical Sketch:

Male - REDACTED

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Education:

A. B. (chemistry and mathematics) Central Methodist College, Fayette, Missouri & Ph. D. (biochemistry under E. A. Doisy) St. Louis University, St. Louis, Missouri & Postdoctoral fellow (NRC) at the California Institute of Technology, & ., under Linus Pauling. Research was concerned with physiochemical study of sickle cell hemoglobin (Hb-S).

Professional Experience:

Creighton University School of Medicine, Omaha, Nebraska,

Professor of Biochemistry and Chairman, Department of Biochemistry, 1961- Research has been concerned with the metabolism and metabolic effects of choline, and serum enzymes especially lecithin: cholesterol acyl-transferase and atherogenesis.

State University of New York Upstate Medical Center,

Syracuse, New York. Instructor of Biochemistry, Department of Biochemistry, 1950-52; Assistant Professor, 1952-56; Associate Professor, 1956-61. Research was concerned with synthesis of antibiotics produced by Pseudomona aerugionosa, biosynthesis of cholesterol, and studies of metabolic efforts of choline antimetabolites.

Society Memberships:

REDACTED REDACTED

Honors and Awards:

Co-winner, Commercial Solvents Corp. Award in Antibiotics (administered by Am. Soc. Bacteriologists), 1952. Listed in Who's Who in America, 1968 -.

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Personal Publications (five most recent)

Wells, I. C., "Hemorrhagic kidney degeneration in choline deficiency", Federation Proceedings 30, 151 (1971).

Wells, I. C., "Release of intracellular enzymes in serum", Canad. J. Biochem. 47, 347 (1969).

Wells, I. C. and Rongone, E. L., "Dietary cholesterol and serum cholesterol esterifying activity in rabbits", Proc. Soc. Exp. Biol. and Med. 127, 1006 (1968).

Wells, I. C. and Hogan, J. M., "Effects of dietary deficiencies of lipotropic factors on plasma cholesterol esterification and tissue cholesterol in rats", J. Nutrition 95, 55 (1968).

Wells, I. C. and Krajeski, J. P., "Hormonal influences on choline concentrations in rat tissues", Endocrinology 82, 693 (1968).

14. First year budget:

A. Salaries (give names or state "to be recruited")
Professional (give % time of investigator(s) even if no salary requested)

	% time	Amount
Medical Technologist	100%	$ 8,000
Junior Technician	100%	6,000
TOTAL		14,000

Technical

Fringe	1,120
Sub-Total for A	15,120

B. Consumable supplies (by major categories)

Blood culture tubes	1,500
Conical centrifuge tubes	750
Disposable microfilters	500
Culture media	2,000
Chemicals	2,000
Other supplies (pipettes, flasts, cuvettes ——)	1,500
Sub-Total for B	8,250

C. Other expenses (itemize)

Travel for sample collection	1,500
Maintenance of equipment	500
Sub-Total for C	2,000
Running Total of A + B + C	25,450 25370

D. Permanent equipment (itemize)

Sub-Total for D	3806
E	3,817
Total request	29,267 29176

E. Indirect costs (15% of A+B+C)

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	15,876	8,500	2,000		3,956	30,332
Year 3	16,669	8,800	2,000		4,120	31,589

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Hereditary Progressive A-V Conduction Defect	HEW 1 R01 HL 15903-01	$35,000	9/1/73 - 8/31/74
Breast Cancer Family Resources	HEW-NO1-CB-33901	95,120	7/1/73 - 6/1/74
Genetics of Cardiac Conduction Defects: Family Studies	Nebraska Heart Association	7,158	7/1/73 - 6/31/74
Order of Eagles	Nebr. Tuberculosis and	10,000	
Isolation and characterization of SRS-A	Respiratory Disease Ass'n.	2,500	7/1/73 - 6/31/74
Lysolecithin in atherogenesis	Nebr. Heart Ass'n.	4,130	7/1/73 - 6/31/74

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Prospective Genetic Studies of Colon and Lung Cancer: Host Environment Considerations	Research Council at Omaha Veterans Administration Hospital	$14,200	9/1/73 - 8/1/75
Additional Clinical Centers for the Multiple Risk Factor Intervention Trial for the Prevention of Coronary Heart Disease	RFP-NHLI-74-1	289,101	9/1/74 - 8/1/75
Carcinoembryonic Antigen in Two Cancer Prone Families	NIH - Ca 15635-01	100,503	9/1/73 - 8/31/76

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Henry T. Lynch, M.D.

Signature [signature] Date 11/7/73

Telephone Area Code REDACTED Extension

Checks payable to

Creighton University

Mailing address for checks

2500 California

Omaha, Nebraska 68178

Responsible officer of institution

Typed Name LeRoy Kozeny

Title Controller

Signature [signature] Date 11/7/73

Telephone Area Code REDACTED Number Extension

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Literature Review

According to presently accepted concepts, the carcinogenic polycyclic aromatic hydrocarbons must be metabolized by certain mixed-function oxidases to reactive intermediates to elicit cell transformation, mutagenicity and cytotoxicity (1).

Aryl hydrocarbon hydroxylase (AHH) is one of these mixed function oxidases and occurs in the microsomal fraction of most tissues of the mouse and other experimental animals investigated (2-4) and probably in most tissues of man (5-8). It is an inducible enzyme since its activity is increased after the administration to animals of a number of different agents, including polycyclic hydrocarbons, drugs, steroids, insecticides and various other substances (9).

Recently, Kouri, et al. (10) have reported a relationship between the inducibility of AHH in mice and the susceptibility to 3-methylcholanthrene induced tumors. However, no correlation could be discerned between sarcomas evoked by 7,12-dimethylbenz(a)anthracene or benzo(a)pyrene and the inducible hydroxylase activity among the same inbred strains of mice. Genetic studies have indicated that inducibility in mice is under the control of a single genetic locus (11-14) and hybridization studies in hamster, mouse, and human cells indicate a closely coupled control mechanism for inducibility and basal AHH activity (15).

Kellermann, et al. (16) have observed that variation in extent of AHH induction in cultured human leukocytes is under genetic control and that the normal white population in the United States can be divided into three distinct phenotypes with low, intermediate and high degrees of inducibility. Two alleles and a single locus appear to be involved with the three groups representing homozygous low and high alleles and the intermediate heterozygote.

The distribution followed the Hardy-Weinberg equilibrium, and gene frequencies of the low and high alleles in this population were 0.717 and 0.283 respectively. Phenotype frequencies were 53%, 37%, and 10%. Family studies included all six possible crosses, and none of the offspring varied from expectations. Huberman and Sachs (17) reached similar conclusions using a different test system.

Kellerman, et al. (18) have recently reported data from a study of fifty patients with bronchiogenic carcinoma which indicate that susceptibility to this disease is associated with higher levels of inducible aryl hydrocarbon hydroxylase activity.

have indicated that inducibility in man is [illegible]

References

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3. Nebert, D.W., Goujon, F.M., Gielen, J.E.: Aryl Hydrocarbon Hydroxylase Induction by Polycyclic Hydrocarbons: Simple Autosomal Dominant Trait in the Mouse, Nature (New Biol.) 236:107-110, 1972.

4. Idem: Genetic Expression of Aryl Hydrocarbon Hydroxylase Induction. III. Changes in the Binding of N-Octylamine to Cytochrome P-450, Mol. Pharmacol. 8:651-666, 1972.

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6. Juchau, M.R., Pederson, M.G., Symms, K.G.: Hydroxylation of 3,4-Benzpyrene in Human Fetal Tissue Homogenates, Biochem. Pharmacol. 21:2269-2272, 1972.

7. Busbee, D.L., Shaw, C.R., Cantrell, E.T.: Aryl Hydrocarbon Hydroxylase Induction in Human Leukocytes, Science 178:315-316, 1972.

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9. Conney, A.H.: Pharmacological Implications of Microsomal Enzyme Induction, Pharmacol. Rev. 19:317-366, 1967.

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12. Nebert, D.W., Gielen, J.E.: Genetic Regulation of Aryl Hydrocarbon Hydroxylase Induction in the Mouse, Fed. Proc. 31:1315-1325, 1972.

13. Thomas, P.E., Kouri, R.E., Hutton, J.J.: The Genetics of Aryl Hydrocarbon Hydroxylase Induction in Mice: A Single Gene Difference Between C57BL/6J and DBA/2J, Biochem. Genet. 6:157-168, 1972.

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16. Kellermann, G., Luyten-Kellermann, M., Shaw, C.R.: Genetic Variation of Aryl Hydrocarbon Hydroxylase in Human Lymphocytes, Amer. J. Hum. Genet. 25:327-331, 1973.

17. Huberman, E., Sachs, L.: Metabolism of the Carcinogenic Hydrocarbon Benzo(a)pyrene in Human Fibroblast and Epithelial Cells, Int. J. Cancer 11:412-418, 1973.

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ship Between Susceptibility to 3-Methylcholanthrene-Induced Subcutaneous

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